COUPLING FACTOR ADENOSINE TRIPHOSPHATASE-COMPLEX OF *RHODOSPIRILLUM RUBRUM*

Isolation of an oligomycin-sensitive Ca^{2+}, Mg^{2+}–ATPase

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1. Introduction

Membranes from various photosynthetic bacteria were found to contain divalent cation-dependent ATPase activities [1–3]. In *Rhodospirillum rubrum* chromatophores an ATPase requiring either Ca^{2+} or Mg^{2+} was observed and its activity was inhibited by oligomycin [4]. Attempts to separate the ATPase from *R. rubrum* membranes were carried out by several groups. Johansson et al. [5] purified a Ca^{2+}-dependent ATPase from an acetone powder of the membranes, whereas Koning and Guillory [6] isolated a Mg^{2+}-dependent ATPase from the supernatant obtained after EDTA sonication in the presence of dithiothreitol. In both cases the isolated water soluble ATPases were insensitive to oligomycin [6,7].

Oligomycin or DCCD-sensitive ATPases have been isolated by detergent treatment of mitochondria [8] and *E. coli* [9] and found to be larger than the water soluble ATPases, since they contained additional hydrophobic membrane components. Such ATPases have not been isolated as yet from any photosynthetic system. The present report described an oligomycin-sensitive ATPase which has been solubilized by Triton X-100 from *R. rubrum* chromatophores and like the membrane bound enzyme could be activated by either Mg^{2+} or Ca^{2+}.

2. Methods

R. rubrum strain S_1 cells were grown as previously described [10]. Harvested cells were washed, broken in a Yeda Press and chromatophores were isolated as outlined by Gromet-Elhanan [11,12]. Bacteriochlorophyll was measured using the in vivo extinction coefficient given by Clayton [13].

ATPase activity was separated from the membranes by treatment with Triton X-100. The chromatophores were suspended at a concentration of 0.24 mg bacteriochlorophyll/ml in a solution consisting 0.21% Triton X-100 and 1 mM Hepes–NaOH, pH 8.0 and incubated for 30 min at room temperature. The suspension was centrifuged at 140 000 × *g* for 2 h and the supernatant contained the ATPase activity. This ATPase could be further purified by a glycerol gradeint [14]. Protein was determined according to Lowry et al. [15].

ATPase activity was followed by the change in pH [16]. The reaction was started by addition of either chromatophores or solubilized enzyme. A linear rate of acid production was recorded for at least 5 min. The amount of ATP hydrolysed was calculated from the decrease in pH.

3. Results

Extraction by Triton X-100 releases an ATPase from *R. rubrum* chromatophores. Since the solubilized enzyme preparation contains Triton, the effect of different Triton concentrations on its activity was tested (table 1). Maximal ATPase activity was obtained at 0.014% Triton X-100, which represents a ratio of 5 mg Triton/mg protein. Higher Triton concentrations resulted in inhibition of the ATPase activity. Similar

Table 1
Effect of increasing Triton X-100 concentrations on the soluble ATPase

Triton X-100 concentration	ATPase activity
(% w/v)	(μmol/mg bacteriochlorophyll/h)
0.007	15.0
0.014	22.0
0.025	10.6
0.055	5.2

The reaction mixture contained, in final vol. 3 ml: 3.3 mM Hepes–NaOH, pH 8.0; 2 mM $MgCl_2$; 4 mM ATP and Triton X-100 to a final concentration as indicated. The reaction was started by addition of soluble enzyme containing 90 μg protein. This amount of enzyme contained enough Triton to give a final concentration of 0.007%.

results were reported in a Triton X-100 extracted mitochondrial ATPase [8]. In all further experiments a constant Triton concentration of 0.014% was used.

The catalytic activity of *R. rubrum* membrane bound ATPase could be induced by either Mg^{2+} or Ca^{2+} [4]. Similarly, the Triton solubilized ATPase exhibits both Ca^{2+}- and Mg^{2+}-dependent activities (figs 1,2,4). A specific ratio of cation to ATP was found to be required for optimal ATPase activity in the Triton solubilized enzyme [14]. These ratios were 1 : 2 for the Mg^{2+}–ATPase and 1 : 1 for the Ca^{2+}–ATPase. Using these ratios the dependence of the catalytic activity on ATP concentration was tested (figs 1,2). The K_m for ATP was found to be 0.3 mM for Mg^{2+}–ATPase (fig.1) and 1.0 mM for the Ca^{2+}–ATPase (fig.2).

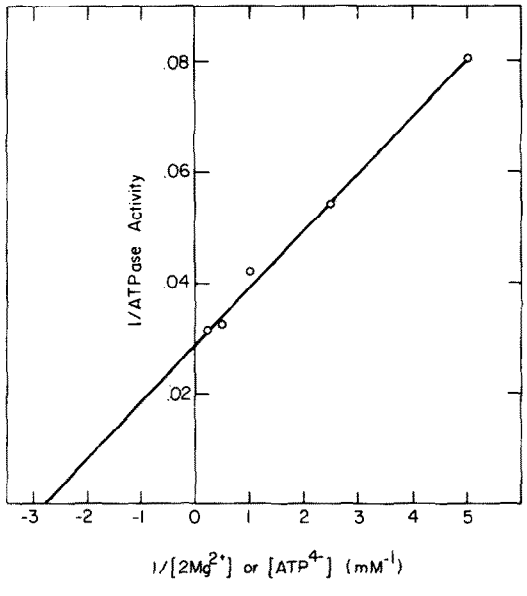

Fig.1. Kinetics of Mg^{2+}-dependent ATPase activity as a function of ATP concentrations. The reaction mixture contained, in final vol. 3 ml: 3.3 mM Hepes–NaOH, pH 8, and changing concentrations of Mg^{2+} and ATP^{4-} at a fixed ratio of 1:2. The reaction was started by addition of soluble enzyme containing 30 μg protein.

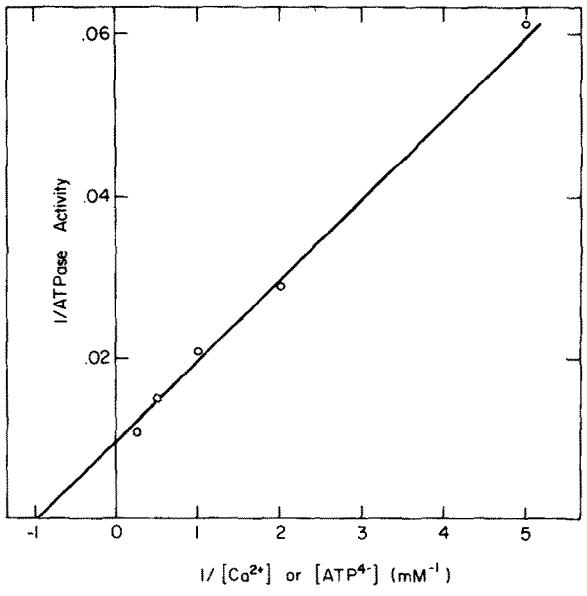

Fig.2. Kinetics of Ca^{2+}-dependent ATPase activity as a function of ATP concentrations. Conditions as described in fig.1, except that a fixed ratio of Ca^{2+} : ATP^{4-} of 1 : 1 was used throughout.

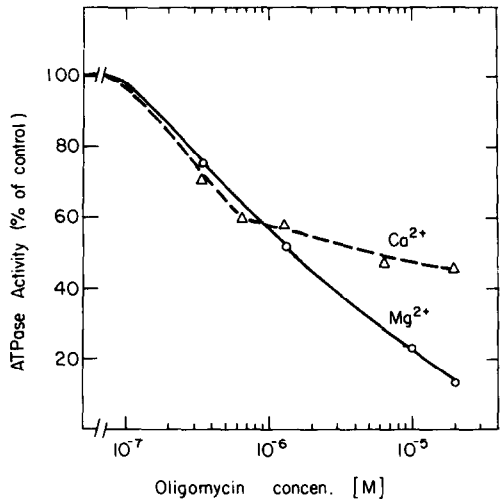

Fig.3. Effect of oligomycin on the membrane bound ATPase. The reaction mixture contained, in final vol. 3 ml: 3.3 mM Hepes–NaOH, pH 8.0; 4 mM ATP, and either 2 mM $MgCl_2$ or 4 mM $CaCl_2$ and oligomycin as indicated. The reaction was started by the addition of chromatophores containing 40 μg of bacteriochlorophyll. Control activities expressed in μmol ATP hydrolysed/mg bacteriochlorophyll/h were for Ca^{2+}–ATPase (Δ) - 76 and for Mg^{2+}–ATPase (○) – 203.

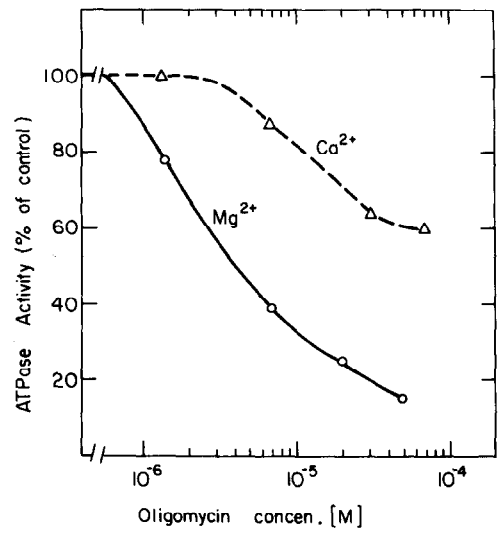

Fig.4. Effect of oligomycin on the Triton solubilized ATPase. Conditions as described in fig.3, except that the reaction was started by addition of soluble enzyme containing 100 μg protein. Control activities expressed in μmol ATP hydrolyzed/mg protein/h were for Ca^{2+}–ATPase (Δ) – 17 and for Mg^{2+}–ATPase (○) – 38.

Studies with *Chromatium* strain D(17) and with chloroplasts [18] led to the suggestion that the substrate for ATPase is a cation–nucleotide complex. If the *R. rubrum* ATPase activity is replotted as a function of the complex concentration (not shown here) a K_m of 0.08 mM for the $(Mg–ATP)^{2-}$complex and 0.3 mM for the $(Ca–ATP)^{2-}$complex is obtained.

The sensitivity of the enzyme to oligomycin was measured both in the bound and in the soluble state, with either Mg^{2+} or Ca^{2+} (figs 3,4). The membrane bound Mg^{2+}-dependent ATPase was 50% inhibited by 1 μM oligomycin. No significant change in the sensitivity of the Mg^{2+}–ATPase to oligomycin was observed after Triton solubilization (fig.4). Increasing oligomycin concentrations inhibited both the bound and the soluble Mg^{2+}–ATPase activities by more than 80%. A different picture was obtained with the Ca^{2+}- and Mg^{2+}–ATPase showed a similar sensitivity to oligomycin (fig.3), whereas in the solubilized form the Ca^{2+}–ATPase was more resistant to oligomycin than the Mg^{2+}–ATPase. A concentration of more than 50 μM oligomycin was required for 40% inhibition of the soluble Ca^{2+}–ATPase (fig.4). At higher oligomycin concentrations a white precipitate appeared in the presence of Ca^{2+} and the inhibition of both bound and soluble activities levelled off.

4. Discussion

The ATPase activity of *R. rubrum* photosynthetic membranes is dependent on divalent cations, with both Ca^{2+} and Mg^{2+} being active [4]. Both activities were also found to be oligomycin-sensitive. The data summarized above demonstrate that Triton X-100 extraction released from *R. rubrum* chromatophores on oligomycin-sensitive Ca^{2+}, Mg^{2+}–ATP. The bound and solubilized Mg^{2+}–ATP exhibit a similar sensitivity to oligomycin, whereas the soluble Ca^{2+}–ATPase is more resistant than the bound one (figs 3,4). The soluble ATPase activities were tested in the presence of 0.014% Triton X-100 (table 1). In the *R. rubrum* enzyme, unlike in the mitochondrial solubilized ATPase [19], this Triton X-100 concentration did not cause loss of oligomycin sensitivity (compare figs 3,4).

The water soluble ATPases, which have been

previously isolated from *R. rubrum* chromatophores were specific for either Ca^{2+} [5] or Mg^{2+} [6] and were oligomycin insensitive. The Triton X-100 solubilized ATPase which is activated by both Mg^{2+} (fig.1) and Ca^{2+} (fig.2) and is oligomycin sensitive (fig.4) is therefore much more similar to the membrane bound ATPase. It has indeed been found to contain more polypeptides subunits than the water soluble ATPase [14]. Further characterization of the Triton X-100 solubilized ATPase complex is now in progress in our laboratory.

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